SHORT NOTE

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Single-step immunoaffinity purification of the neuropeptide sericotropin using polyclonal antibodies towards the synthetic N-terminal fragment of the molecule

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Abstract. An immunoaffinity chromatography of the neuropeptide sericotropin using rabbit polyclonal antibodies towards the 16 amino acids synthetic fragment of the molecule was used for the purification of the peptide from *Galleria mellonella* larval brains. The method employed CNBr-activated Sepharose and the isolated IgG fraction of the corresponding antiserum. The purity of the peptide was proven by means of HPLC and immunoblotting techniques.

Introduction

Sericotropin is a secretory peptide that was identified in the wax moth (*Galleria mellonella*; Lepidoptera: Pyraliade) larvae (Kodrík & Sehnal, 1991; Michalik et al., 1992). It is released from the brain under both in vitro and in vivo conditions and stimulates silk production by elevating RNA synthesis in posterior part of the silk glands. Its molecular weight in brain extracts is about 10 kDa (Kodrík & Sehnal, 1991). Sericotropin was purified by use of FPLC and HPLC techniques and was partially sequenced (Kodrík et al., 1994). With an oligonucleotide probe designed on the basis of the sequence, sericotropin clones were identified in a brain-specific cDNA library. The sericotropin gene deduced from these results encodes for 133 amino acids, 16 of which were identified as the signal peptide (Kodrík et al., 1995; V.A. Filippov unpublished data). Recently, we found that sericotropin belongs to a group of insect lipocalin-like proteins (Filippov et al., 1995).

In this report we describe fast and effective purification of sericotropin by immunoaffinity chromatography to receive more pure material for physiological studies.

Materials and Methods

Immunoaffinity chromatography

A synthetic fragment of sericotropin (16 AA, Leu-Thr-Asp-Glu-Gln-Lys-Glu-Lys-Leu-Lys-Lys-His--Arg-Ser-Glu-Cys), prepared on the basis of the reported results was conjugated to keyhole limpet haemocyanin and used for production of polyclonal antibodies in rabbits. The specificity of the antiserum was proven on Western blot (Kodrík et al., 1995). This antibody was used for isolation of further sericotropin material from 3,200 *G. mellonella* larval brains using immunoaffinity chromatography.

Protein A-Sepharose CL-4B (Pharmacia) was used to isolate the IgG fraction from the crude antiserum (0.6 g Protein A-Sepharose per 2 ml of the antiserum) following routine protocols. An immunoadsorbent was prepared by coupling the isolated IgG fraction on CNBr-activated Sepharose (Pharmacia) at a ratio of 30 mg IgG per g dry gel. After removal of uncoupled IgG by repeated rinses with the application (PBS, 150 mM K_2HPO_4 and 0.5 M NaCl, pH 7.6) and elution (50 mM glycine and 150 mM NaCl, pH 3.0) buffers, the crude brain extract (Fig. 1A) containing the sericotropin antigen was applied to the immunoadsorbent by recycling the extract over the column 10 times for 1h. After rinsing the column to baseline

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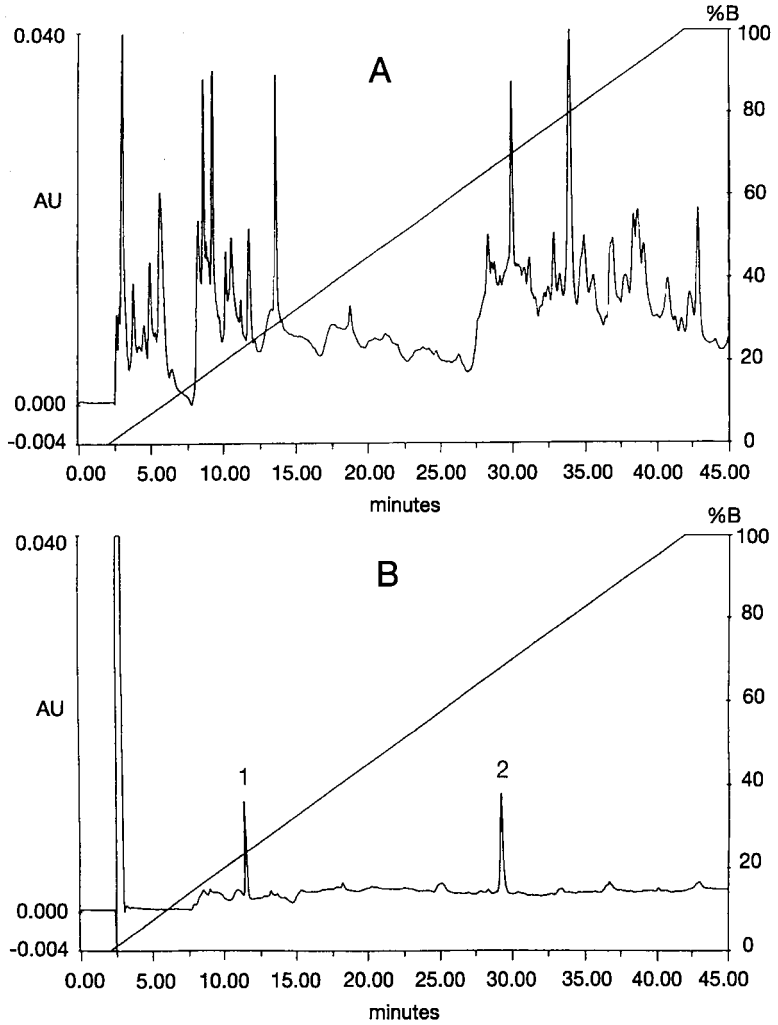

Fig. 1. RP HPLC evaluation of the one-step immunoaffinity purification protocol of sericotropin. A – the profile of the starting material corresponding to 50 brains from *G. mellonella* larvae (water soluble material); B – the affinity-purified sericotropin (peak 2) corresponding to 300 brains equivalent; peak 1 could be a rest of Ig (see text).

absorption (280 nm), the adsorbed material was eluted with the elution buffer pH 2.5. PBS was added to the eluate for neutralization.

Reversed phase (RP) HPLC and Western blotting

The purity of the putative sericotropin preparation was assessed by RP HPLC (Merck-Hitachi D-6000 chromatography system, LiChrospher WP 300 RP-18 column, 250 × 4 mm, particles 5 μm) with a gradient from 0 to 100% B in 38 min (from 2 to 40 min) (A = 0.11% TFA in water; B = 0.1% TFA in 60% acetonitrile) at a flow rate of 1 ml/min and U.V. detection at 210 nm.

After SDS electrophoresis according to Laemmli (1970) on a 20% polyacrylamide gel, the proteins were electroblotted onto a nitrocellulose membrane (Towbin et al., 1979). For immunodetection, the

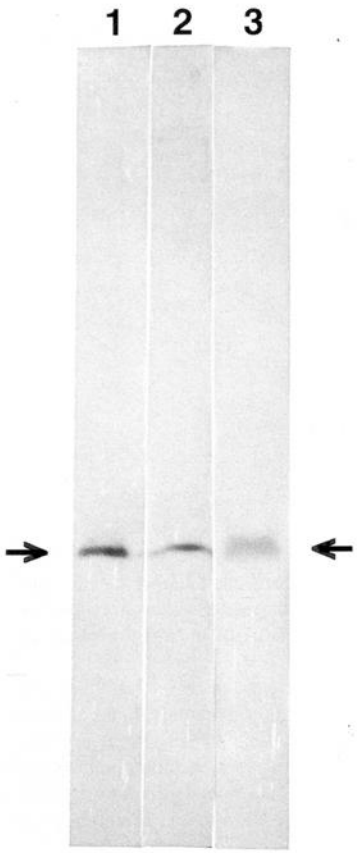

Fig. 2. Immunoblotting of sericotropin material after SDS-PAGE (see text); arrows indicate sericotropin positive material: 1 – crude brain material from *G. mellonella* larvae (brain equivalent = 4); 2 – affinity-purified sericotropin (brain equiv. = 10); 3 – peak number 2 from RP HPLC test of sericotropin purity (Fig. 1B) (brain equiv. = 300).

membrane was rinsed in the following solutions (Johnson et al., 1984): (1) 5% skimmed milk (Difco) in PBS-Tween 20, 1 h, (2) PBS-Tween 20, 5 × 5 min, (3) primary antiserum against the sericotropin in PBS-Tween 20, 1 : 1000, 1 h, (4) PBS-Tween 20, 5 × 5 min, (5) swine anti-rabbit IgG conjugated with horseradish peroxidase (Sevac, Praha), 1 : 1,000, 1 h, (6) PBS-Tween 20, 5 × 5 min, (7) 0.01% hydrogen peroxide and 0.025% o-diaminobenzidine in 0.1 M tris pH 7.6, 1–3 min, (8) distilled water.

Results and Discussion

Upon reversed phase HPLC of the immunoaffinity-purified material, a single peak in the expected area (elution at 70% B) was obtained (Fig. 1B, peak number 2). The identity of peak 1 is yet to be investigated but may theoretically be an Ig contamination from the immunoadsorbent. In the Western blotting protocol (Fig. 2), HPLC peak number 2 was labelled by the polyclonal antiserum at the same migration position as was sericotropin in crude brain extracts (lane 1) and in affinity-purified sericotropin (lane 2).

These results show that sericotropin can be purified in a fast and simple protocol by taking advantage of the specificity of the antiserum against its synthetic N-terminus.

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